

ORIGINAL ARTICLE

Novel *IFT140* variants cause spermatogenic dysfunction in humans

Xiong Wang¹ | Yan-wei Sha² | Wen-ting Wang³ | Yuan-qing Cui¹ | Jie Chen¹ | Wei Yan¹ | Xiao-tao Hou⁴ | Li-bin Mei² | Cui-cui Yu⁵ | Jiahui Wang³ 

¹Department of Reproductive Medicine, The Affiliated Yantai Yuhuangding Hospital of Qingdao University, Yantai, Shandong 264000, China

²Department of Reproductive Medicine, Xiamen Maternal and Child Care Hospital, Xiamen, Fujian, China

³Central Laboratory, The Affiliated Yantai Yuhuangding Hospital of Qingdao University, Yantai, Shandong, China

⁴Pathology Department, Guangzhou KingMed Center for Clinical Laboratory Co. Ltd, Guangzhou, Guangdong, China

⁵Department of Anesthesiology, The Affiliated Yantai Yuhuangding Hospital of Qingdao University, Yantai, Shandong, China

Correspondence

Li-bin Mei, Department of Reproductive Medicine, Xiamen Maternal and Child Health Hospital, Xiamen, Fujian, China.
Email: meilibinxm@163.com

Cui-cui Yu, Department of Anesthesiology, Yantai Yuhuangding Hospital, Yantai, Shandong, China.
Email: yhdyyhhf@126.com

Jiahui Wang, Central Laboratory, Yantai Yuhuangding Hospital, Yantai, Shandong, China.
Email: jiahui.wang@outlook.com

Funding information

Medicine and Health Science Technology Development Project of Shandong Province, Grant/Award Number: 2016WS0704; Natural Science Foundation of Shandong Province, Grant/Award Number: ZR2017LH012; Science and Technology Project of Fujian Province, Grant/Award Number: 2017D011

Abstract

Background: The intraflagellar transport protein 140 homolog (IFT140) is involved in the process of intraflagellar transport (IFT), a process that is essential for the formation and maintenance of most eukaryotic cilia and flagella. Variants IFT140 have been reported to account for ciliopathy but association with male fertility has never been described in humans. Here we report the identification of two novel variants of IFT140 which caused spermatogenic dysfunction and male infertility.

Methods: Whole-exome sequencing was performed in a 27-year-old infertile man presented with severe oligozoospermia, asthenozoospermia, and teratozoospermia (OAT) without other physical abnormality. Sanger sequencing was used to verify gene variants in the patient, his healthy brother, and their parents. Morphology and protein expression in the patient's sperm were examined by transmission electron microscopy (TEM) and immunofluorescence staining. Function of gene variants was predicted by online databases.

Results: Compound heterozygous variants of IFT140: exon16: c.1837G > A: p.Asp613Asn and exon31: c.4247G > A: p.Ser1416Asn were identified in the patient, both of which showed autosomal recessive inheritance in his family, and had extremely low allele frequency in the population. Morphological abnormalities of the head, nucleus, and tails and the absence of IFT140 from the neck and mid-piece of the patient's spermatozoa were observed. Mutation Taster database predicted a high probability of damage-causing by both variations.

Conclusion: This study for the first time reported IFT140 variants that cause infertility in humans.

KEYWORDS

genetic variation, IFT140, intraflagellar transport, male infertility, spermatogenic dysfunction

*Xiong Wang, Yan-wei Sha equally contributed to this paper.

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1 | INTRODUCTION

Studies have shown that 8% of reproductive male population suffers fertility disorders. It is estimated that there are at least 20 million infertile men worldwide (Boivin, Bunting, Collins, & Nygren, 2007; Esteves, 2013). With the in-depth study of the mechanism of gene-regulated spermatogenesis, the value of genetic variation in etiological diagnosis of male infertility has been increasingly emphasized (Krausz, Escamilla, & Chianese, 2015).

Intraflagellar transport (IFT) is a bidirectional motility along axonemal microtubules that is essential for the formation (ciliogenesis) and maintenance of most eukaryotic cilia and flagella. The process of IFT involves movement of large protein complexes called IFT particles, which are composed of about 20 proteins that could be divided into two complexes: the anterograde IFT complex A and the retrograde IFT complex B (Cole et al., 1998). Intraflagellar transport protein 140 homolog (IFT140) is one component of the IFT complex A. The humans *IFT140* (OMIM accession number: 614,620), located on chromosome 16p13.3, has 40 exons. *IFT140* is expressed in a variety of organs, with high RNA expression level in the testis, some endocrine tissues (e.g., pituitary gland, thyroid gland, and adrenal gland), and central nervous tissues (e.g., cerebellum, caudate, and hippocampus) as reported by the Bgee database (<https://bgee.org/>). IFT140 protein was composed of 1,462 amino acids, containing five WD repeat domains and nine tetratricopeptide repeat (TPR) domains. Intracellularly, IFT140 protein was reported to locate on the cilium basal body (Hull et al., 2016; Perrault et al., 2012) and centrosome (Schmidts et al., 2013).

This study reported a male patient who suffered primary infertility due to severe oligozoospermia, asthenozoospermia, and teratozoospermia (OAT). Through combined use of high-throughput whole-exome sequencing and Sanger sequencing, we identified compound heterozygous variants in his *IFT140*. This is the first reporting case of IFT140 mutation causing infertility in humans.

2 | MATERIAL AND METHODS

2.1 | Ethical compliance

This study was approved by the Ethics Committee of the Yantai Yuhuangding Hospital. Written informed consent was obtained from all participants.

2.2 | Semen sample preparation

Fresh semen was collected from the patient and his brother after 3–7 days of sexual abstinence and tested at least twice in accordance with the WHO guidelines (World Health Organization, 2010). Liquefied samples were washed with

mHTF (Irvine Scientific, Santa Ana) at 37°C and processed as previously described (Wang et al., 2017).

2.3 | Peripheral whole blood DNA extraction

DNA was extracted from peripheral whole blood samples using the QiAmp Blood DNA mini Kit according to the manufacturer's instructions (Qiagen, Hilden, Germany).

2.4 | Whole-exome sequencing and data analysis

Whole-exome sequencing was performed by Annoroad Gene Technology (Beijing, China) using the AgilentV6 60M Exon Targeting Sequence Enrichment System (Agilent, USA). Data were analyzed for variations in the *IFT140* (Genbank accession No. NM_014714.4). Frequency of the observed mutations in the general population was assessed using 1,000 Genomes project (<http://www.1000genomes.org/data>), Exome Variant Server/NHLBI Exome Sequencing Project (ESP6500) (<http://evs.gs.washington.edu/EVS/>), and Exome Aggregation Consortium (ExAC) (<http://exac.broadinstitute.org/>). Analysis of mutations for their probable impact on protein function was carried out using SIFT (<http://sift.jcvi.org/>), Polyphen-2 (<http://genetics.bwh.harvard.edu/pph2/>), Mutation Taster (<http://www.mutationtaster.org/>), and SNPs&Go databases (<http://snps.biofold.org/snps-and-go/>). Functional protein association was predicted with the STRING (<https://string-db.org/>), GeneMANIA (<http://genemania.org/>), and HitPredict (<http://hintdb.hgc.jp/hp/>) databases.

2.5 | Variant confirmation and cosegregation

Polymerase chain reaction (PCR) and Sanger sequencing were employed to validate the variants and to determine their inheritance and cosegregation. Exon 16 and 31 of *IFT140* were amplified from genomic DNA using the following primers: IFT140_E14F: 5'-TGTGGACTTGGTGACACTGGG-3'; IFT140_E14Rseq: 5'-CAACTCCAATCTACCCACTGCC-3'; IFT140_E29Fseq: 5'-GCATCCCCTCATCACCACA TT-3'; IFT140_E29R: 5'-GTCGCGTATTCCAACACA GACAT-3'. The sequencing of PCR products was performed bidirectionally using the ABI3500 sequencer (Life technology, USA). Analysis of sequences was carried out using Mutation Surveyor (SoftGenetics).

2.6 | Transmission electron microscopy (TEM)

TEM was performed as previously described (Wang et al., 2017).

2.7 | Immunofluorescence

Prepared spermatozoa were smeared onto polylysine-coated slides and fixed with methanol at -20°C for 20 min. Test slides were blocked with 2% casein in PBS and stained with the rabbit anti-IFT40 (17460-1-AP, Proteintech, USA) and mouse anti-acetylated tubulin (66200-1-Ig, Proteintech, USA) primary antibodies and anti-mouse FITC-conjugated (ZF-0516, Zsbio, China) or anti-rabbit DsRed-conjugated (ZF-0512, Zsbio, China) secondary antibodies. Nuclei were stained with DAPI. Slides were mounted with ProLong Gold Antifade Reagent (Invitrogen, USA) and examined using a LEICA TCS SPE confocal microscope (LEICA, Germany).

3 | RESULTS

3.1 | Novel IFT40 variants identified in an infertile male patient

The patient was a 29-year-old male, 175 cm in height, weighed 100 kg, and was in good general health without obvious discomfort. He had been married for 2 years without child. Within the past year, he had regular sex intercourse of two to three times per week with vaginal ejaculation. The patient's elder brother was 34 years old, whose wife had naturally conceived and given birth to a healthy child.

Physical examination of the patient identified no abnormality in bilateral epididymis and seminal ducts, and no palpable bilateral varicocele. Blood sex hormone levels were: follicle-stimulating hormone 10.26 mIU/ml, luteinizing hormone 8.38 mIU/ml, testosterone 2.12 ng/ml, estradiol 14.07 pg/ml, prolactin 7.01 ng/ml, and progesterone 0.642 ng/ml. The patient had normal chromosome karyotype, without Y chromosome microdeletions. No abnormality was identified in his seminal vesicle gland, prostate, bilateral testis, epididymis, and spermatic vein by ultrasonography. No other neurological, skeletal, developmental abnormalities, and no renal failure or retinopathy were found.

The patient presented severe OAT. Routine semen analysis showed a sperm concentration of 0–2/HPF with very few active spermatozoa. Sperm morphology analysis found only 0.5% normal spermatozoa, and the vast majority of spermatozoa had irregular head and aberrant neck (Figure 1a,b). The patient's sperm was examined with TEM analysis in comparison to normal sperm. Result showed that normal spermatozoa usually had long cone-shaped heads, long strip-shaped tails, and abundant mitochondria on both sides of the tails (Figure 1c). In contrast, the spermatozoa from the patient had abnormal head shape, separation of acrosome from nucleus, irregular morphology of sperm nucleus, short or swollen tails

with local bulging, abnormal morphology, and distribution of mitochondria in sperm tails (Figure 1d,e). Semen sample of the patient's healthy brother was also examined, which showed normal semen parameters. Whole-exome sequencing was performed for the patient, which identified two point mutations in the *IFT140*: exon16: c.1837G > A: p.Asp613Asn and exon31: c.4247G > A: p.Ser1416Asn.

3.2 | IFT40 variants showed family aggregation

Blood samples were collected from the patient, his elder brother, and their both parents. Sanger sequencing was performed to confirm their genotype of IFT40 and cosegregation. The results showed that the patient carried heterozygous alleles of both variants, each of which was inherited from one of the parents. Heterozygosity of IFT40: c.1837G > A was confirmed in the patient's father and IFT40: c.4247G > A was found in the patient's brother and mother (Figure 2a). Both variants showed family aggregation in the patient's pedigree (Figure 2b).

3.3 | Bioinformatics analysis of IFT140 variants

In silico analysis of the IFT40 variants suggested that both variants had extremely low allele frequency in the population (Table 1). Especially, IFT40: c.4247G > A was only identified in the total ExAC database. Orthology analysis showed that both mutation sites were highly conserved across mammalian species as well as Zebrafish (Figure 3). Bioinformatics analysis through the Mutation Taster database predicted a high probability of damage-causing by both variations, while Polyphen-2, SIFT, and SNPs&GO analyses suggested that both variations were neutral (Table 1). Protein interaction analysis by STRING database predicted interaction of IFT40 with IFT122, IFT43, WDR35 (IFT121), WDR19 (IFT144), and TTC21B that belong to the IFT complex A and with IFT88, IFT81, IFT80, IFT52, and IFT27 that belong to IFT complex B. Analysis by GeneMANIA and HitPredict also identified TULP3, a binding partner of IFT complex A, and RANBP2 as high-confidence interactive protein of IFT40.

3.4 | Expression of IFT140 in spermatozoa

We used immunofluorescent staining to examine the expression of IFT40 in the spermatozoa of the patient comparing to those of normal male as control. Result showed that IFT40 protein was completely absent in the patient's spermatozoa, but was evidently expressed in the neck and mid-piece of normal spermatozoa (Figure 4).

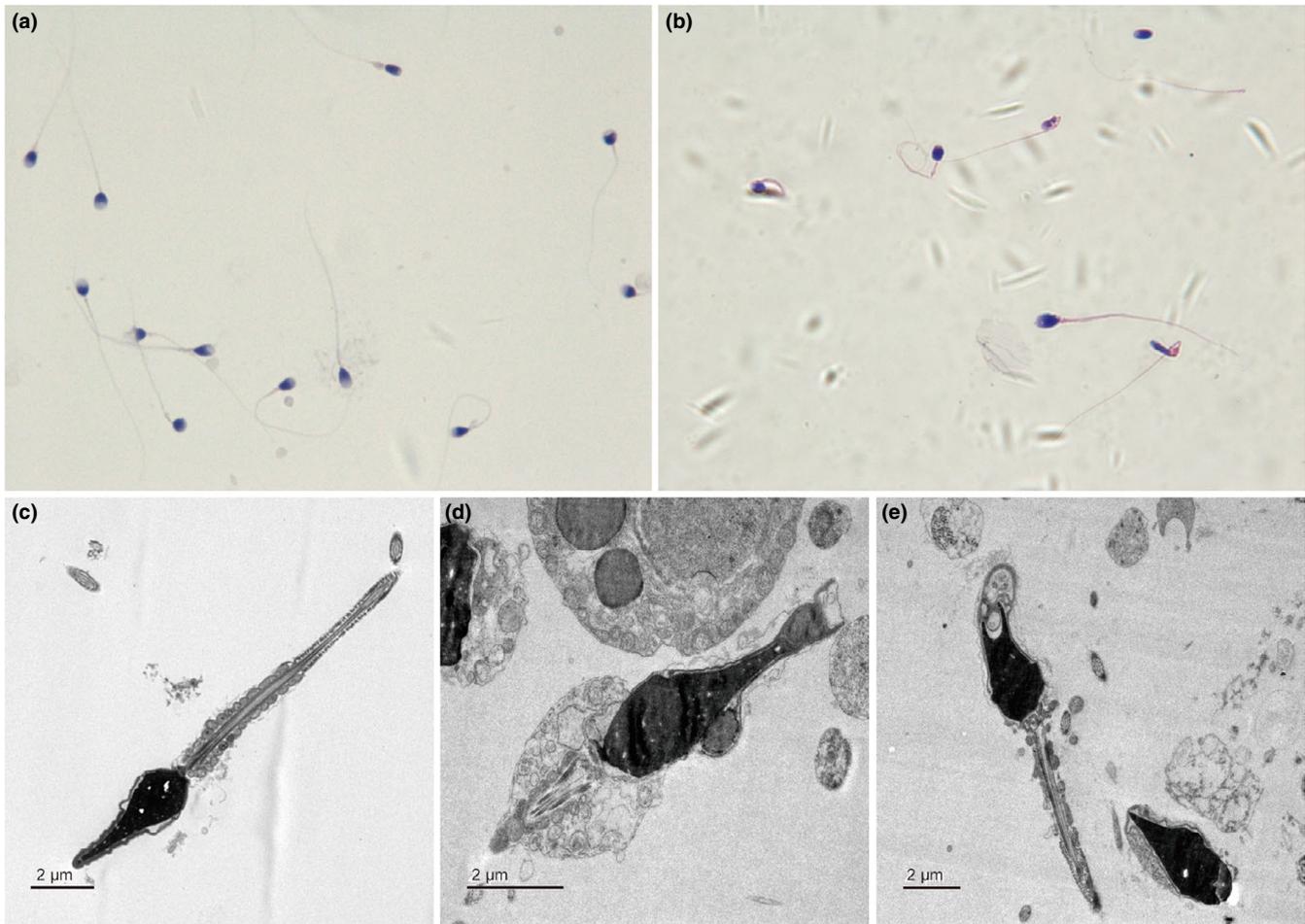


FIGURE 1 Morphological analysis of spermatozoa from (a) a normal person and (b) the patient by Papanicolaou staining, $\times 400$ magnification. Transmission electron microscopy images of spermatozoa from (c) a normal person and (d, e) the patient

4 | DISCUSSION

During spermatogenesis in male mammals, spermatogonia develop into mature spermatozoa through extremely complex cell differentiation processes, which involve thousands of genes. The normal expression of these genes is the basis for maintaining fertility in male mammals (Schultz, Hamra, & Garbers, 2003). Clinically, abnormality in semen parameters is a common cause of male infertility, such as nonobstructive azoospermia and severe OAT. These abnormalities are largely related to the loss of expression or abnormal expression of genes regulating testicular spermatogenesis. Animal models and genetic screening studies have confirmed genetic factors to be the common cause of spermatogenesis disorders (Cannarella, Condorelli, Duca, Vignera, & Calogero, 2019). For example, pathogenic variations in *FANCM* (Kasak et al., 2018), *MEIOB* (Gershoni et al., 2019), *RNF212*, *STAG3* (Riera-Escamilla et al., 2019), *TEX11*, *TEX12*, *TEX14*, and *TEX15* (Boroujeni et al., 2018) genes may cause nonobstructive azoospermia in male humans. A previous study by our

group also identified a male with cryptozoospermia caused by a highly pathogenic nonsense mutation in *TEX15* (Wang et al., 2018). The diverse clinical manifestations may be due to individual differences. The motility of sperm is closely related to the ultrastructure of sperm. In recent years, great progress has been made in the study of genes related to sperm ultrastructure, such as *AK7* (Lores et al., 2019), *ARMC2* (Coutton et al., 2019), *CEP135* (Sha et al., 2017), *CFAP43*, *CFAP44* (Tang et al., 2017), *CFAP69* (He et al., 2019), *CFAP251* (Li et al., 2019), *DNAH1* (Wang et al., 2017), *FSIP2* (Martinez et al., 2018), *QRICH2* (Shen et al., 2019), *SPEF2* (Liu, Lv, et al. 2019; Liu, He, et al., 2019), and *TTC21A* (Liu, Lv, et al. 2019; Liu, He, et al., 2019). Variations in these genes can affect spermatogenesis, leading to multiple flagella deformities or sperm loss, which manifest as severe teratozoospermia and severe asthenozoospermia. In addition, the correlation between genetic factors and idiopathic oligoasthenospermia has also received increasing attention (Fruhmesser et al., 2013; Vucic et al., 2018).

In this study, semen examinations showed that the patient had severe oligozoospermia and asthenozoospermia, which

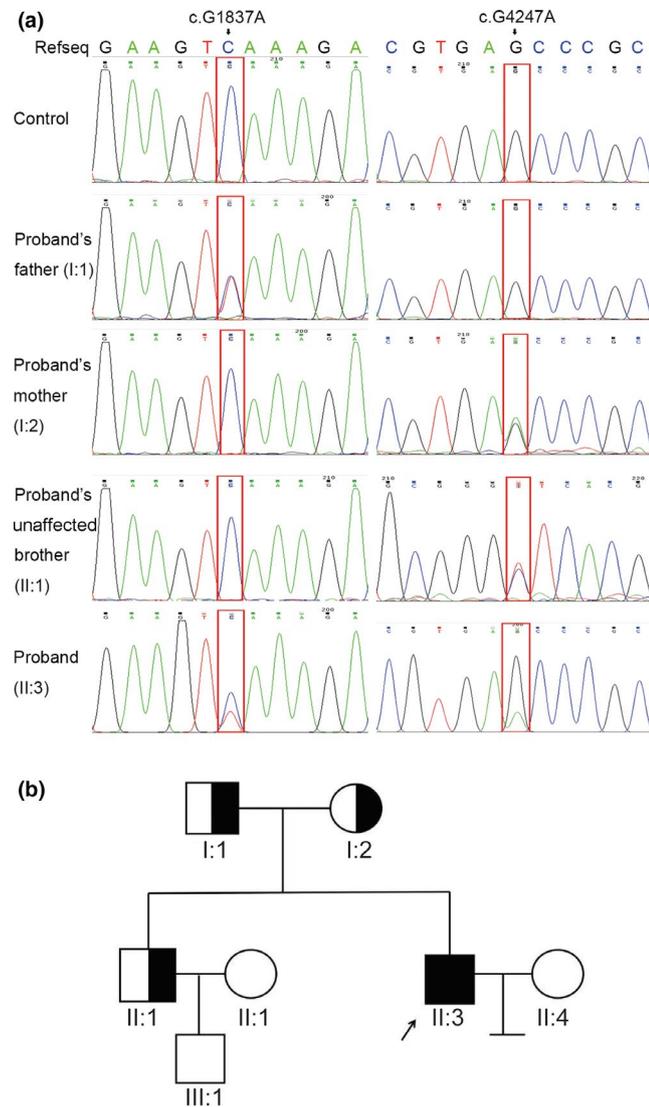


FIGURE 2 (a) Sanger sequencing alignment of the patient and his close relatives. (b) Family tree of the patient

suggested spermatogenic dysfunction. Compound heterozygous mutations of c.1837G > A and c.4247G > A were identified in *IFT140* of the proband, while his brother was a heterozygous carrier of *IFT140*: c.4247G > A. Each of the two variations originated from one parent. Both the proband and his brother were born naturally by their parents. The brother and his spouse naturally gave birth to a healthy child. These suggested cosegregation of the variations and phenotype in the pedigree conformed to autosomal recessive inheritance.

A number of *IFT140* variants have been reported to be associated with ciliopathy in humans, namely the Jeune syndrome (asphyxiating thoracic dystrophy) and Mainzer-Saldino syndrome (MSS) (Helm et al., 2017; Perrault et al., 2012; Schmidts et al., 2013) (Table 2). Jeune syndrome is a rare genetic disorder that affects cartilage and bone development, causing dwarfism, breathing difficulties, and renal

TABLE 1 In silico analysis of *IFT140* mutations

Gene mutation	Amino acid change	1000G_ALL ^a	1000G_EA ^b	ExAC (total) ^c	ExAC_EA ^d	ESP6500_siv2_ALL ^e	ESP6500_siv2_EA ^f	Polyphen-2 ^g	SIFT ^h	Mutation Taster ⁱ	SNPs&GO ^j
c.1837G > A	p.Asp613Asn	0.0002	0.001	0.00002471	0.0003	0	0	Benign (0.215)	Tolerated (0.169)	Disease causing (1)	Neutral (0.041)
c.4247G > A	p.Ser1416Asn	NA	NA	0.00003213	0	0	0	Benign (0.001)	Tolerated (0.621)	Disease causing (0.983)	Neutral (0.147)

^aFrequency of variation in total of 1,000 Genomes database (A Deep Catalog of Human Genetic Variation).

^bFrequency of variation in East Asian population of 1,000 Genomes database.

^cFrequency of variation in total of ExAC database.

^dFrequency of variation in East Asian population of ExAC database.

^eFrequency of variation in total of ESP6500 database.

^fFrequency of variation in East Asian population of ESP6500 database.

^gPolyphen-2 (<http://genetics.bwh.harvard.edu/pph2/>). Prediction Scores range from 0 to 1 with high scores indicating probably or possibly damaging.

^hSIFT, that is, Sorting Intolerant From Tolerant (<http://sift.jcvi.org/>). Scores vary between 0 and 1. Variants with scores close or equal to 0 are predicted to be damaging.

ⁱMutation Taster (<http://www.mutationtaster.org/>). The probability value is the probability of the prediction, that is, a value close to 1 indicates a high "security" of the prediction.

^jSNPs&GO (<http://snps.biofold.org/snps-and-go/>). Probability: disease probability (if > 0.5 mutation is predicted disease).

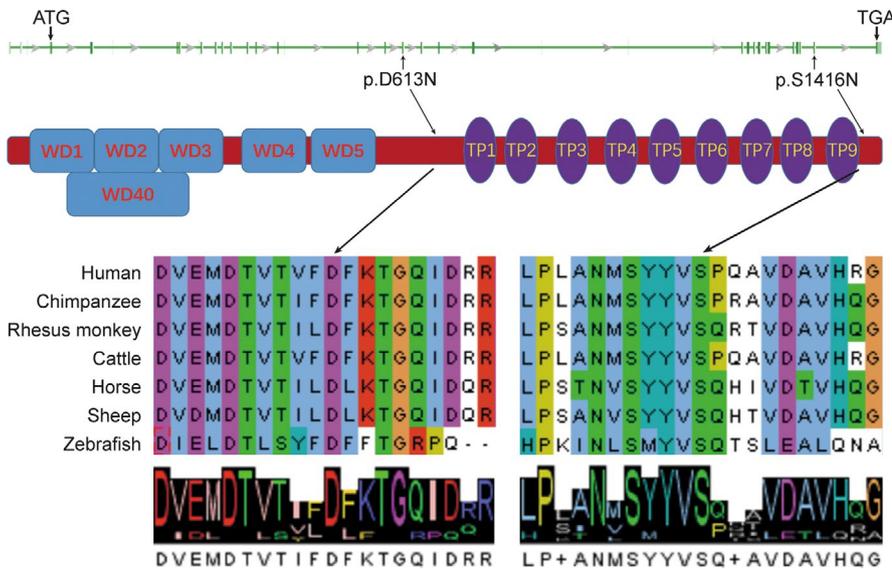


FIGURE 3 Schematic diagram showing the location of mutations in the *IFT140* and protein of the patient, and the conserved sequences of the mutation points across various species

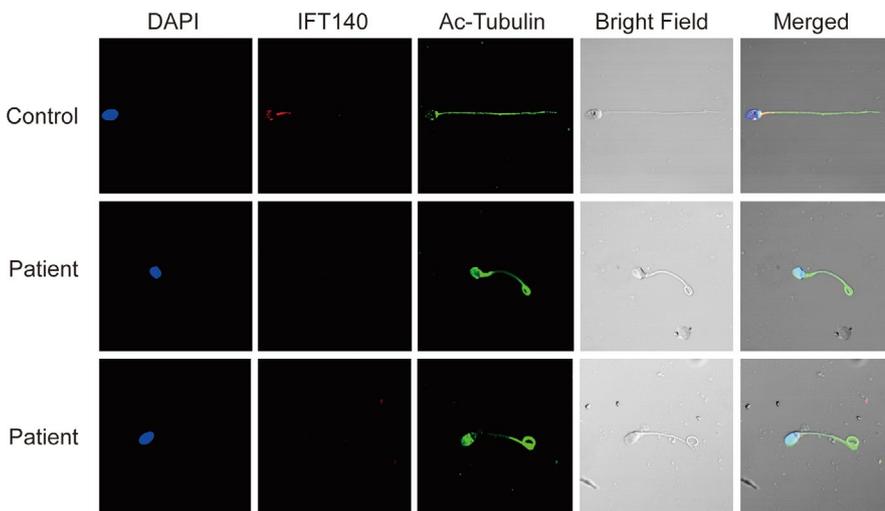


FIGURE 4 Immunofluorescence staining of the spermatozoa of a normal person and the patient

failure (de Vries et al., 2010). MSS patients are characterized by craniofacial and skeletal abnormalities, delayed motor development, and are often associated with cystic dysplastic kidneys, dextrocardia, and agenesis of corpus callosum (Badano, Mitsuma, Beales, & Katsanis, 2006). Hull *et al.* reported six biallelic variants of *IFT140* that caused retinitis pigmentosa (RP) while not affecting the patient's nervous and skeletal systems and renal function (Table 2) (Hull et al., 2016). Disruption of *Ift140* in *Chlamydomonas* and *Trypanosoma* has also been reported to result in the formation of short flagella (Absalon et al., 2008; Zhu et al., 2017).

The two variants identified in this study have not been reported to be associated with human diseases. Bioinformatics analysis through the Mutation Taster database predicted a high probability of damage-causing by both variations, while Polyphen-2, SIFT, and SNPs&GO analyses suggested that both variations were neutral. However, a recent animal study (Zhang et al., 2018) found that IFT140 was a key regulator of male fertility and normal spermatogenesis in mice. *Ift140*

was highly expressed in the testicular tissues of normal mice. Male germ cell-specific knockout of *Ift140* in mice resulted in spermatogenic dysfunction, abnormal sperm morphology (amorphous heads, short/swollen flagella, and other distorted shapes as well as flagellar vesicles), and defective sperm motility, which ultimately led to male infertility. These observations were in accordance with the manifestations of the patient in this study, which serves as a strong evidence for the pathogenicity of the two identified *IFT140* variants. The human *IFT140* is homologous to mouse *Ift140*, and the phenotypes of mutations are largely the same. Therefore, we speculate that *IFT140* plays a key role in spermatogenesis in male mammals.

The mechanism of how IFT140 variants affect spermatogenesis is not fully understood. The patient's spermatozoa displayed bulged flagellar tips, as observed in *Ift140*-deficient mice. This is a typical phenotype of retrograde trafficking deficiency (Hirano, Katoh, & Nakayama, 2017; Zhang et al., 2018). As the patient displayed almost the same conditions with the

TABLE 2 Summary of IFT140 variant-related diseases

Disease	Major clinical symptoms	Genotype of IFT140 variants	Reference
Jeune syndrome (asphyxiating thoracic dystrophy)	Short stature, small thorax, end-stage renal failure under 13 years of age, retinal dystrophy	c.1380delC (p.Asn460Lysfs28*)/ c.874C > T (p.Val292Met), c.1565G > A (p.Gly522Glu)/ c.874C > T (p.Val292Met), c.454C > T (p.Leu152Phe)/c.454C > T (p.Leu152Phe), c.2278C > T (p.Arg759*)/?	Schmidts et al. (2013)
	Developmental delay, nystagmus, short thorax with short ribs, trident limbs, PCSE, nonspecific nephritis	c.2399 + 1G > T (splice)/c.634G > A (p.Gly212Arg)	Perrault et al. (2012)
Mainzer-Saldino syndrome (MSS)	Short stature, small thorax, end-stage renal failure under 13 years of age, retinal dystrophy	c.2399 + 1G (splice)>T/c.4078T > C (p.Cys1360Arg), c.418G > A (p.Gly140Arg)+c.800A > G (p.Glu267Gly)/c.490G > T (p.Glu164*)	Schmidts et al. (2013)
	Dysgraphia, Reading difficulties, Adjustment disorder, developmental delay; high myopia, nyctalopia, retinitis pigmentosa, rod-cone dystrophy; acute-onset renal failure; hepatomegaly	c.634G > A (p.Gly212Arg)/c.634G > A (p.Gly212Arg)	Helm et al. (2017)
	Early-onset retinal dystrophy, PCSE, and renal disease, some with nonovert renal disease	c.634G > A (p.Gly212Arg)/c.3916dup (p.Ala1306Glyfs*56), c.932A > G (p.Tyr311Cys)/c.857_860del (p.Ile286Lysfs*6), c.2399 + 1G > T (splice)/c.1990G > A (p.Glu664Lys), c.1990G > A (p.Glu664Lys), c.1990G > A (p.Glu664Lys), c.699T > G (p.Ile233Met)/ c.699T > G (p.Ile233Met), c.1565G > A (p.Gly522Glu)/-, c.874G > A (p.Val292Met)/-, c.1727G > A (p.Arg576Gln)/-, c.489C > T (p.Gly163Gly)+c.488_491del (p.Glu164Thrfs * 10)/-	Perrault et al. (2012)
Retinitis pigmentosa (RP)	Developmentally normal, with no apparent skeletal, neurological abnormalities or renal failure at age 13 to 67 years	Bi-allelic mutations of c.1451C > T (p.Thr484Met), c.2399 + 1G > T (splice), c.2815T > C (p.Ser939Pro), c.998G > A (p.Cys333Tyr), c.1021G > A (p.Ala341Thr), c.1422_23insAA (p.Arg475Asnfs*14)	Hull et al. (2016)
Primary infertility	Spermatogenic dysfunction, developmentally normal, no apparent skeletal, neurological abnormalities, no renal failure, no retinal dysfunction	c.1837G > A (p.Asp613Asn)/ c.4247G > A (p.Ser1416Asn)	This paper

Note: PCSE: phalangeal cone-shaped epiphyses.

male germ cell-specific *Ift140*-deficient mice, we assumed that the IFT140: Asp613Asn and IFT140: Ser1416Asn mutations disrupt key protein-binding sites or secondary structures of IFT140, leading to attenuated protein function in the spermatogenesis, but not affecting its functions in other systems.

In conclusion, compound heterozygous variants of *IFT140*: exon16: c.1837G > A: p.Asp613Asn/exon31: c.4247G > A: p.Ser1416Asn might cause spermatogenic dysfunction and infertility in humans. The results indicated the importance of ciliogenesis and IFT in male reproduction.

This is the first reporting case of IFT140 mutation causing infertility in humans. Unfortunately, there was only one patient identified with IFT140 variant in this study. Future identification of more cases might provide better evidence for the involvement of IFT140 in humans spermatogenesis.

ACKNOWLEDGMENTS

This study was financially supported by the Medicine and Health Science Technology Development Project of Shandong Province (Grant no. 2016WS0704), the Natural Science Foundation of Shandong Province (Grant No. ZR2017LH012), and the Science and Technology Project of Fujian Province (Grant No. 2017D011).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Jiahui Wang  <https://orcid.org/0000-0002-0867-8612>

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How to cite this article: Wang X, Sha Y-W, Wang W-T, et al. Novel *IFT140* variants cause spermatogenic dysfunction in humans. *Mol Genet Genomic Med*. 2019;7:e920. <https://doi.org/10.1002/mgg3.920>